

# PATENT SPECIFICATION

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## (54) DETOXIFICATION OF NUTRIENT PLANT MATERIALS

(71) We, UNILEVER LIMITED, a company organised under the laws of Great Britain, of Unilever House, Blackfriars, London, E.C.4, England, do hereby declare the invention which was communicated from Hindustan Lever Limited, of Hindustan Lever House, 165-166 Backbay Reclamation, Bombay 20, India, a company organised under the laws of India, for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the detoxification of certain nutrient plant materials, in particular those nutrient plant materials which are toxic by virtue of the presence in them of one or more toxic saponins, so as to fit them for use in animal feeds.

One example of such a plant material is alfalfa (lucerne); it has for example been reported that feeding chicks with a diet containing as little as 0.2% by weight of alfalfa saponin inhibits their growth. Another example is defatted mowrah meal, which, although it ordinarily contains about 50% carbohydrate, 8—13% residual fat and 15—17% protein, and is thus a potential ingredient of animal feeds, cannot be used for that purpose owing to its high content (5% or more) of toxic saponins. Of the very large amounts of defatted mowrah meal which in India are a by-product of the extraction of edible oil (known as mahua butter, mowrah oil or bassia fat) from the seeds of *Madhuca latifolia* (syn. *M. indica*, *Bassia latifolia*, family Sapotaceae), most is used as manure, fish- or worm-poison or fuel.

The present invention is concerned with detoxifying such saponin-containing nutrient plant materials, so as to fit them for, or improve their fitness for, use in animal feeds.

According to the invention, nutrient plant material having a content of toxic saponins is detoxified by treating it with a hydrolysing agent under conditions such as to bring about hydrolysis of the saponins to sapogenins. By contrast with the parent saponins, the sapogenins are free from glycosidic units at the

C3 hydroxyl group in ring A of the steroid structure, are water-insoluble, have practically no surface-activity, and are in substance non-toxic. The sugars liberated from the saponins as a result of hydrolysis add to the nutritional value of the product.

The hydrolysing agent employed may be an enzyme, but is preferably an acid, particularly a mineral acid, such as sulphuric acid or hydrochloric acid, the latter being preferred. Hydrolysing conditions are preferably provided by heating the plant material with a dilute mineral acid, preferably to at least 75°C. Reaction is conveniently carried out by heating the system to and maintaining it at boiling point.

Excess acid in the product of hydrolysis can be neutralised by addition of an alkali metal or alkaline earth metal hydroxide, such as sodium or potassium hydroxide or calcium or magnesium hydroxide, or ammonia. The preferred agent is sodium hydroxide.

Water can be removed from the neutralised product by filtration or centrifugation, but it is best removed by evaporating the product to dryness. This has the advantage of avoiding loss of soluble nutrient materials.

The product can be used as an ingredient of poultry and ruminant feeds, at levels of up to 10% and 20% by weight respectively.

The invention is further illustrated by the following Example.

### EXAMPLE

5 kg of powdered defatted mowrah meal (saponins content 7%) was heated under reflux for 3 hours with 20 litres of 2% hydrochloric acid. There was at first much foaming, but this gradually subsided and eventually stopped altogether. The mixture of acid and meal, with its saponin content now hydrolysed to non-surface-active sapogenins, was cooled, and to it was added 7 litres of water, followed gradually and with stirring by a solution of 450 g sodium hydroxide in 1 litre of water.

The mixture was then poured onto a shallow pan and dried in a hot air oven for 24 hours. The dried treated meal weighed 5.3—5.4 kg, most of the increase in weight from the original

5 kg being due to the formation of sodium chloride at the stage of neutralisation of the acid used to bring about hydrolysis of the saponins.

5 Chromatographic tests were carried out as follows.

10 10 g of each of the untreated and acid-treated defatted mowrah meal was heated for 15 minutes with 50 ml of absolute alcohol and filtered. The filtrates were made up to 50 ml with more absolute alcohol. 0.1 ml of each of these was spotted on 3 MM Whatman paper, and ascending chromatograms were developed using n-butanol:ethanol:water (6:2:3) and then stained by the Liebermann-Burchard reagent (equal amounts of acetic anhydride and concentrated sulphuric

acid). An intense pink violet spot at Rf 0.6 and a very faint spot of the same colour at Rf 0.94 appeared with the extract of the untreated meal, whereas with the extract of treated meal there was no coloured spot in the region Rf 0.6. There was instead a strong pink violet spot in the region Rf 0.94. This confirmed that the saponin had been converted by acid hydrolysis to the faster moving and lighter sapogenin.

The product obtained by the process of the Example was incorporated in a conventional poultry feed to form feed A as shown below, and its effect on the growth rate and feed conversion efficiency of chicks was compared with that of feeds B (control) and C (containing untreated mowrah meal).

Ingredient	Percentage by weight		
	Feed A*	Feed B*	Feed C*
Treated mowrah meal	5.0	—	—
Untreated mowrah meal	—	—	5.0
Rice bran	13.0	18.0	13.0
Bone meal	0.3	0.3	0.3
Extracted groundnut cake	25.0	25.0	25.0
Extracted rice bran	12.5	12.5	12.5
Fish meal	3.5	3.5	3.5
Limestone	0.6	0.6	0.6
Maize	37.0	37.0	37.0
Molasses	3.0	3.0	3.0
Salt	0.1	0.1	0.1

\* Each feed contained an identical conventional (very small) quantity of a conventional vitamin/trace metal/antibiotic premix, which is not shown.

There was no statistically significant difference in the performance of chicks fed on feeds A and B, showing that the treated meal can be safely used at a level of at least 5% by weight of the feed. With feed C, more than 90% of the birds had died by the end of the fifth week from the start of feeding.

Post-mortem macroscopic and microscopic examination of the tissues of laboratory rats (even those of f2 generation) fed on diets containing the treated meal at 30% level did not reveal any abnormalities.

#### WHAT WE CLAIM IS:—

1. A method of detoxifying nutrient plant material having a content of toxic saponins, in which the material is treated with a hydro-

lysing agent under conditions such as to bring about hydrolysis of the saponins to sapogenins.

2. A method according to Claim 1, in which the hydrolysing agent is an acid.

3. A method according to Claim 2, in which the acid is a mineral acid.

4. A method according to Claim 3, in which the nutrient plant material is heated with dilute hydrochloric acid.

5. A method according to any one of Claims 1 to 4, in which excess acid in the product of hydrolysis is neutralised by an alkali metal or alkaline earth metal hydroxide.

6. A method according to Claim 5, in which water is removed from the neutralised product by evaporating the product to dryness.

7. A method according to any one of Claims 1 to 6 in which the nutrient plant material is defatted mowrah meal.
9. Defatted mowrah meal when detoxified by the method of any one of Claims 1 to 7.
- 5 8. Nutrient plant material when detoxified by the method of any one of Claims 1 to 7.

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